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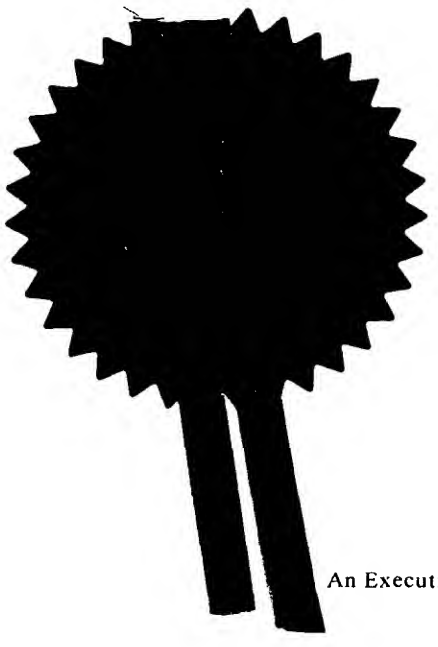

Form 1/77

The Patent Office

17MAR99 E433068-1 D01631
P01/7700 0.00 - 9905998.2

1/77

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form.)

17 MAR 1999

The Patent Office
Cardiff Road
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Fee: £0

1. Your reference

40644 / JMD

2. Patent application number
(The Patent Office will fill in this part)

9905998.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

CeNeS Limited
Compass House
Vision Park
Chivers Way
Histon
Cambridge, CB4 4ZR

Patents ADP number *(if you know it)*

7313777002

If the applicant is a corporate body, give the country/state of incorporation

United Kingdom

4. Title of the invention

Interface Patch Clamping

5. Full name, address and postcode in the United Kingdom to which all correspondence relating to this form and translation should be sent

Reddie & Grose
16 Theobalds Road
LONDON
WC1X 8PL

Patents ADP number *(if you know it)*

91001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application *(if you know it)*	Date of filing *(day/month/year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patent Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document.

Continuation sheets of this form	
Description	11
Claim(s)	3
Abstract	0
Drawing(s)	8

10. If you are also filing any of the following, state how many against each item.

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	0
Request for preliminary examination and search *(Patents Form 9/77)*	0
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature: Reddie & Grose

Date: 16 March 1999

12. Name and daytime telephone number of person to contact in the United Kingdom: J M DAVIES 01223-360350

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INTERFACE PATCH CLAMPING

Introduction

The present invention provides a novel development of the conventional patch clamp technique. This novel technique is referred to as the interface patch clamp method.

Voltage gated ion channels are potential targets for a considerable range of novel treatments in a variety of disease states. The development of the patch clamp technique has provided a powerful method for the study of ion channel function and pharmacology in whole cells. However, while the patch clamp technique provides a definitive method for the investigation and screening of drugs with potential activity on voltage gated ion channels, the technique is currently highly dependent on the skill of the operator and tends to be very slow for drug screening. The present invention provides a method for increasing the rate at which compounds may be screened for ion channel blocking/agonist activity using the patch clamp technique. The method can retain the essential features of the conventional patch clamp recording system while facilitating automation of the major time-consuming components of the technique.

Background: Conventional Patch Clamp

The success of the patch clamp technique is derived from the ability to form "tight" (i.e. high resistance: Giga Ohm) electrical seals between an area of the cell membrane (the Patch) and the tip of a pipette. The patch clamp pipette is usually made from glass. The formation of the G-seal is dependent on the profile of the top of the pipette, and is enhanced by the application of suction to

the interior of the pipette. The requirements for the formation of the G-seals are well established and the process is usually monitored electrically by display of the current pulse recorded in response to a small voltage step applied throughout seal formation. After formation of a G-seal, the area of membrane under the pipette may be disrupted to obtain whole cell voltage clamp recording mode.

The sequence of events leading to successful G-seal formation and whole cell recording mode using pre-formed patch pipettes is as follows:

1. Selection of a suitable cell.

2. The patch pipette is positioned approximately 50 microns above the cell.

3. The pipette is lowered until the cell surface is deformed by the pipette tip.

4. Negative pressure is applied to the interior of the pipette until a G-seal is formed between the pipette tip and the cell membrane.

5. Whole cell recording mode is established by the application of further negative pressure which disrupts the cell membrane in the area under the pipette tip.

Steps two and three are slow and require considerable manual dexterity and a high level of operator skill. Visualisation of the cells and the patch pipette requires the use of a high quality microscope and, in order to position the pipette, a high quality three axis

micromanipulator with sub-micron resolution in each axis is required.

Summary of the Invention

According to the invention, interface patching can utilise a patch pipette of conventional type. Cells are supported on a liquid/air interface at one end of a capillary tube (e.g. made of glass, polyethylene or other suitable material). The axis of the patch pipette is in line with the axis of the tube so that the pipette tip can be manipulated into the opening of the tube where the cells are supported at the air/liquid interface. The capillary tube or the patch pipette can be mounted onto a single axis manipulator. Only one manipulator is required and this may be used to move either the patch pipette or the capillary tube. Whole cell recording mode is established as follows:

6. A layer of cells is established at the interface between the extracellular physiological solution (the liquid in which the cells are suspended) and air by dipping the capillary tube into a suspension of cells. The density of cells in the suspension must be sufficient to provide a sufficient number of cells to form a layer of cells at the interface.

7. Electrical contact with the extracellular solution is established via a non-polarizable electrode (e.g. an Ag/AgCl wire) and the tube is mounted either to a fixed clamp or single axis manipulator.

8. A patch pipette is provided which can be filled with electrolyte solution.

9. The patch pipette is mounted concentrically with the capillary tube either via a single axis manipulator or fixed clamp (if the capillary tube is to be moved). The pipette filling solution is connected via the non-polarizable electrode to the headstage of a conventional patch clamp amplifier. The pipette holder allows suction to be applied to the pipette interior.

10. Cell attached patch mode of recording is established by bringing the pipette tip in contact with the interface by moving the pipette and the capillary tube respectively together along the single mounting axis (e.g. either by moving the pipette towards the tube and interface or vice versa). On entry into the interface the movement of the pipette and capillary tube together is stopped and the pipette current is offset to zero on the patch clamp amplifier. The resistance of the pipette increases when the pipette contacts one of the cells at the air/liquid interface. Suction is then applied to the interior of the pipette and the pipette and capillary tube are moved closer together until the pipette tip is located inside the capillary tube.

Initial seal formation between the pipette tip and the cell may also be assisted by the application of gentle suction during entry of the pipette into the interface.

A G-seal is formed between the patch pipette tip and the cell membrane by the application of further suction to the interior of the pipette and monitoring the pipette resistance.

11. Following the formation of cell attached patch mode, the suction is released, pipette current is offset to zero and a holding voltage applied to the pipette (e.g. -60mV).

12. A whole cell recording is obtained by the application of further suction to the pipette interior until the whole cell recording mode is established in conventional manner.

According to this invention it is preferred that the capillary tube should be mounted in an upright orientation (i.e. essentially vertically) with the air/liquid interface at the downward end of the tube.

This has the advantage that suspended cells will tend to "sediment" naturally to the downward end of the tube and be collected there in a layer. The layer will preferably be several cells deep and loosely packed. Thus according to the invention the pipette tip may be moved upwardly relative to the air/liquid interface at the tube end (either by moving the pipette or the tube along the single axis) so as to come into contact with a cell in the layer at the interface. The relative density or concentration of cells at the interface compared to the density in the bulk of the liquid in the tube ensures a high probability that a cell can be collected on the tip without the need for visualisation of the operation and without the need for multidirectional manipulation of the tip/cell positional relationship. Surprisingly it has been found that G-seal formation between the cell and the pipette can occur without pressing the cell against a solid substrate.

Where the arrangement is intended to operate with the pipette in an upright orientation (i.e. essentially

vertically) with the tip uppermost and pointing upwardly, the pipette should be constructed so as to prevent the filling electrolyte solution flowing out and being lost. This may be achieved for example by use of a custom-made mounting assembly and/or by shaping the pipette body to prevent loss of filling solution (e.g by bending the pipette shaft into a U- or J- shape).

The invention is illustrated by way of example in the accompanying figures in which:

Figure 1a shows a capillary tube containing a suspension of cells; and
Figure 1b shows the cells having formed a layer at the air/liquid interface at one end of the capillary tube;
Figure 2 shows a general arrangement of the interface patch clamp recording equipment with moveable capillary tube;
Figure 2a shows an Apparatus for Interface Patch Clamping with drug/compound application;
Figure 3 shows the cell attached to the patch pipette ready for recording mode.
Figure 4 shows drug/compound addition during interface patch clamp recording: start position;
Figure 5 shows drug/compound addition during interface patch clamp recording: extracellular solution added to dish and dish moved down;
Figure 6 shows drug/compound addition during interface patch clamp recording: solution in dish brought into contact with interface region; and
Figure 7 shows drug/compound addition during interface patch clamp recording: capillary raised above surface of solution in dish.

Referring to figure 1a; a capillary tube (1) of appropriate size can pickup and hold a liquid sample (2) containing cells (3) in suspension. The sample can be picked up simply by dipping the tube end into a suitable bulk liquid reservoir. The liquid in the tube forms an air/liquid interface (4) at the tube end (5). The cells are initially distributed throughout the liquid relatively evenly.

Referring to figure 1b; with the tube in an upright essentially vertical orientation, the cells tend to sediment and to pack loosely together at the lower end of the tube by the tube end to form a layer (6) several cells deep. It will be appreciated by those skilled in the art that the density and depth of the cell layer can be determined by such factors as the cell concentration in the original suspension, the sedimentation time, the relative density of the cells and the liquid etc. It will also be appreciated that means could be devised to encourage or assist cells to migrate from the liquid towards the air/liquid interface rather than or as well as relying on gravitational sedimentation alone. The figure also shows the top of a patch pipette 8 pointing upwardly towards the interface.

Referring to figure 2; an arrangement is shown in which a single axis manipulator is used to move a capillary tube 1 held in a clamp (7) relative to a fixed patch pipette (8) held in a clamp (9). It will be apparent to those skilled in the art that this could be reversed so that the pipette is moved and the tube is fixed. The figure shows the tube clamped in a linear bearing sliding block (10) attached to a motorised single axis manipulator (11). The manipulator should be controlled preferably by computer in order to allow the motion of the manipulator to be varied by

feedback from the patch clamp amplifier. The patch pipette is provided with a connection (12) to a conventional headstage. The system is also provided with a source of variable suction under the control of the patch clamp amplifier/computer.

In figure 2a an arrangement is shown in which additional electromechanical micromanipulators have been added. The micromanipulator labelled (13) is for moving the glass capillary under automated or manual control. A second micromanipulator (14) moves the dish for drug application up and down the glass capillary. A third micromanipulator (15) moves a modified pipette holder to provide electrical contact with the pipette and a means of applying suction to the interior of the pipette. Rotational bases (16 and 17) allow the pipette holder to be moved in and out of the recording area and rotation of the pipette through 180 degrees for filling with pipette solution.

The figure also shows additional features, namely; a pipette holder (18); a patch clamp headstage (19); and a dish holder (20).

A version of the apparatus is envisaged in which patch pipettes will be loaded and filled automatically under software control. It is envisaged also that the loading of capillary glass into the apparatus and the filling with cell suspension will also be automated.

Referring to figure 3; a G-sealed cell 3 is shown held on the tip of the patch pipette 8 and positioned within the entrapped liquid volume in the tube.

Cell attached patch and whole cell (voltage clamp) recording may then be carried out.

The invention described herein has a number of significant features:

- Visualisation of the pipette and the cell is not required.
- Novel recording configuration that would not be considered as obvious.
- Surprisingly G-seal formation occurs without pressing the cell against a hard substrate.
- Cells form a layer at the solution-air interface.
- G-seal formation may be achieved using electronic feedback alone.
- There is no requirement for optical recognition/feedback.
- The system can be automated.
- Multiple recording capillaries and pipettes may be employed in order to allow recordings to be made simultaneously from many cells.

In order to use the invention for screening compound (e.g. for ion channel blocking/agonist activity) the compound of interest needs to be applied to the cell attached to the patch pipette. It will readily be appreciated that this could be achieved in different ways, for example by adding the compound to the extracellular liquid in the capillary tube either before or after G-seal formation. One additional advantage of the invention is that the liquid in the tube could be arranged in layers (e.g. containing different compounds or different concentrations of compounds) and the single axis manipulator could then be used to physically move and position a cell on a pipette tip into a chosen layer (e.g. by moving the G-sealed cell on the tip further up the tube away from the air/liquid interface at one tube end).

A further example of how the effects of compounds may be studied is illustrated in figures 4 to 7.

Figure 4 shows a capillary (1) containing the cell suspension (2) and patch pipette (8) in the recording position for whole cell recording from a cell at the pipette tip. In addition, the capillary tube has been inserted through a hole (21) made in a dish (22) (e.g. 35mm plastic culture dish or similar). The dish is made of a material with hydrophobic properties and the hole allows the dish to be raised and lowered along the axis of the capillary by means of a micromanipulator (14).

Fig 5 shows the dish after it has been filled with extracellular physiological solution (23), which may contain the drug to be studied, or the drug may be added at a later stage. Surprisingly, if the fluid level in the dish is low, leakage through the hole does not occur because the tendency to leak is counterbalanced by:

1. The surface tension of the water
2. The attraction of the water/solution to the glass capillary

After adding the solution to the dish, it is lowered in the direction of the arrow.

Fig 6 shows the solution in the dish in contact with the end of the glass capillary and the patch pipette. The dish and the capillary are now raised simultaneously (arrows A and B) in order to position the pipette tip/cell within the layer of liquid in the dish. If drug is present in the dish at this point and the capillary and dish were moved upwards rapidly, this would constitute a rapid application system particularly useful for the study of agonist responses that desensitise.

Fig 7 shows the effect of raising the capillary so that it is not in contact with the liquid in the dish. The pipette tip/cell remains immersed in the external solution layer in the dish. The solution may be exchanged readily by perfusion of the dish and this allows multiple drug additions and dose response curves to be obtained while recording from the one cell.

It will be readily appreciated by those skilled in the art that:

1. The stability of recording using the interface patch clamp technique may be superior to that of conventional patch clamping. The greater stability of interface patch clamping is because the cell is held by the patch pipette alone. In conventional patch clamp recordings the cell is held by the patch pipette and a solid substrate and vibration tends to move the pipette relative to the substrate causing loss of the G-seal. The interface patch clamp is, in contrast to conventional patch clamp apparatus, relatively insensitive to vibration during drug application.
2. This method of drug application could be applied to a plurality of recording pipettes/capillaries and form the basis for a high throughput electrophysiological assay system. It will readily be appreciated that the Interface Patch Clamp technique could be used with multiple pipettes and multiple capillaries in a manner in which each pipette enters its respective aligned capillary either individually in sequence or all together. Although not currently preferred, a single pipette could be used which is caused to enter more than one capillary sequentially. Multiple patch clamp recordings could be made either sequentially or simultaneously, depending on the application.

Claims

1. A method for providing a cell attached to the tip of a patch clamp pipette and having a high resistance (Giga Ohm) electrical seal between an area of the cell membrane and the tip, which includes the steps of:
i) providing a capillary tube containing a suspension of cells in a liquid;
ii) causing the formation of a layer of cells at one end of the capillary tube at the interface between the air and the liquid in which the cells are suspended;
iii) bringing the tip of the patch clamp pipette into contact with the interface by moving one or both of the pipette and the tube respectively together along a common axis of movement;
iv) contacting the tip with a cell in the cell layer at the interface; and
v) causing attachment of the cell to the tip.

2. A method according to claim 1 in which the liquid in which the cells are suspended is an extracellular physiological solution.

3. A method according to claim 1 in which the layer of cells is several cells deep and loosely packed.

4. A method according to claim 1 in which the layer of cells is formed by mounting the capillary tube in an essentially upright orientation and allowing the suspended cells to sediment to the downward end of the tube to collect there in a layer.

5. A method according to claim 1 in which the capillary tube is mounted essentially upright with the interface at a lower open end of the tube and the pipette is mounted essentially upright with the tip upwardly pointing.

6. A method according to claim 1 in which the capillary tube and pipette are concentrically mounted with the capillary tube in a fixed position and the pipette movable along the common axis.

7. A method according to claim 1 in which the capillary tube and pipette are concentrically mounted with the pipette in a fixed position and the capillary tube movable along the common axis.

8. A method according to claim 1 wherein gentle suction is applied to the pipette during contact with the interface end during the step of contacting the tip with a cell.

9. An apparatus for carrying out the method of any preceding claim which is a computer controlled apparatus including the following elements:
i) a patch clamp amplifier;
ii) a source of variable suction for a patch clamp pipette under the control of the patch clamp amplifier;
iii) a holder for a capillary tube to be mounted vertically;
iv) a holder for a patch clamp pipette to be mounted vertically in the same axis as the capillary tube in an inverted orientation with the tip pointing upwardly;

v) a manipulator for controlling relative movement of the capillary tube and pipette along a common axis of movement under feedback control from the patch clamp amplifier and allowing for the tip of the pipette to enter a downwardly facing end of the capillary tube.

10. An apparatus according to claim 9 which includes an array of a multiplicity of capillary tubes and an array of a multiplicity of pipettes.

FIG 1a

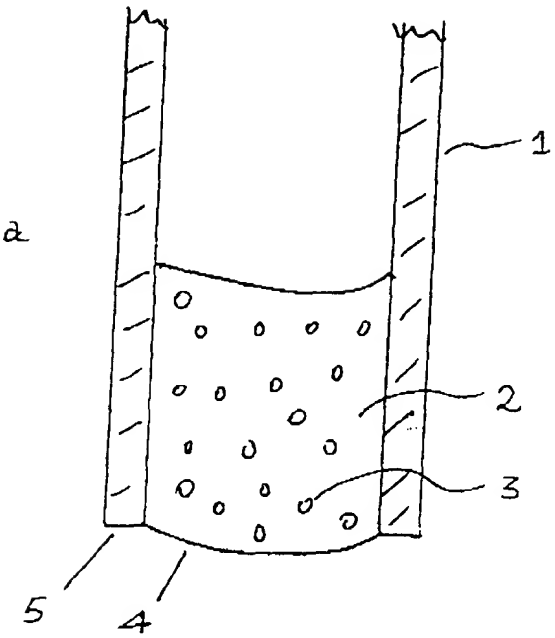


FIG 1b

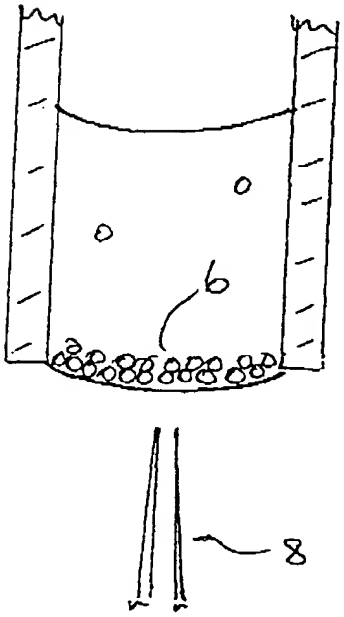
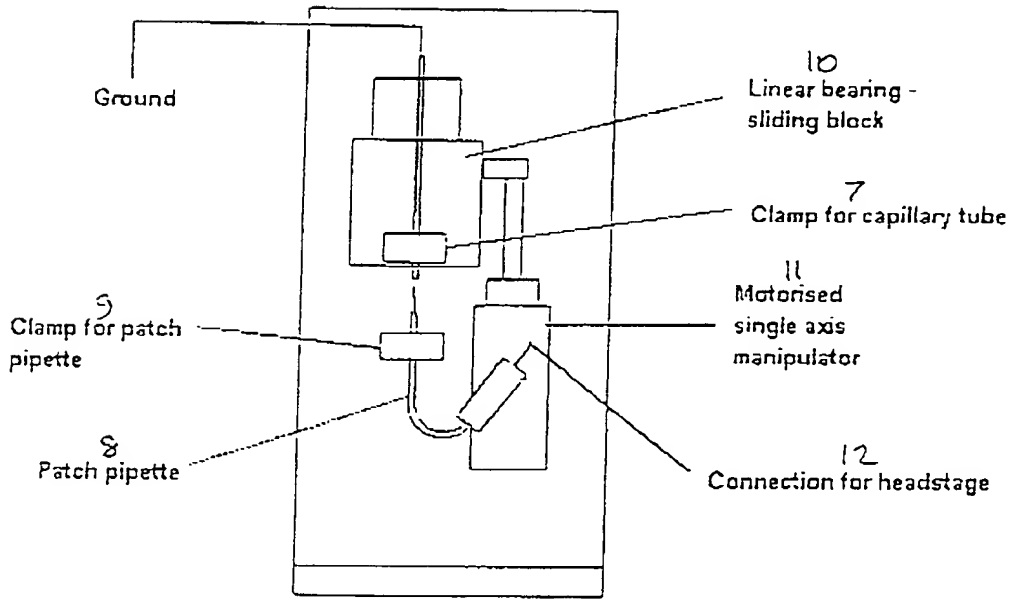


FIG 2


Ground
10
Linear bearing - sliding block
7
Clamp for capillary tube
11
Motorised single axis manipulator
9
Clamp for patch pipette
8
Patch pipette
12
Connection for headstage

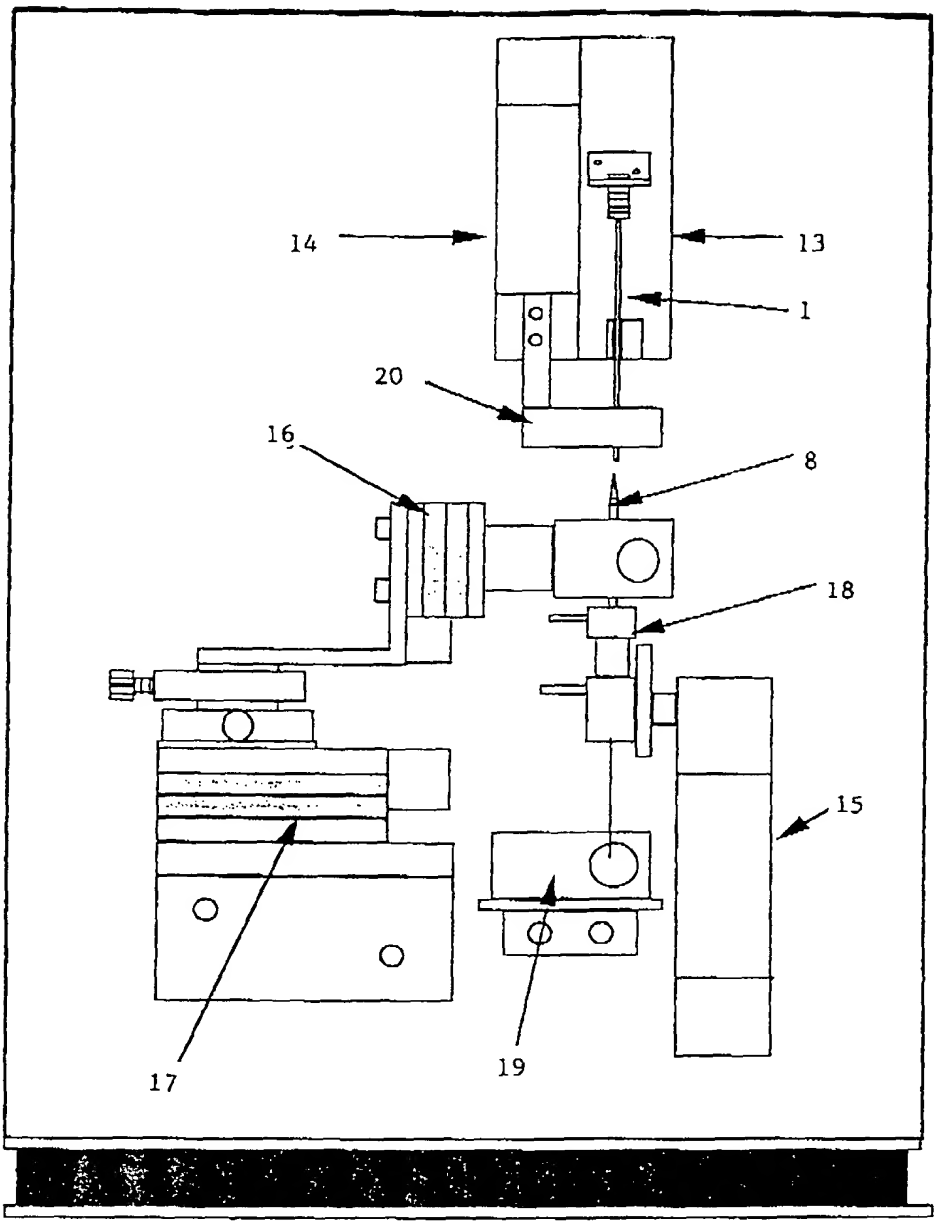
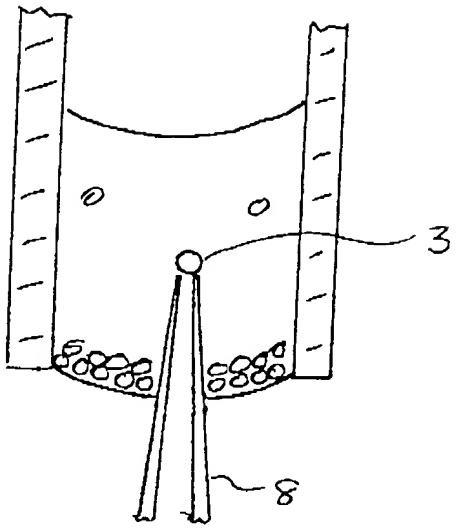


FIGURE 2a

FIG 3



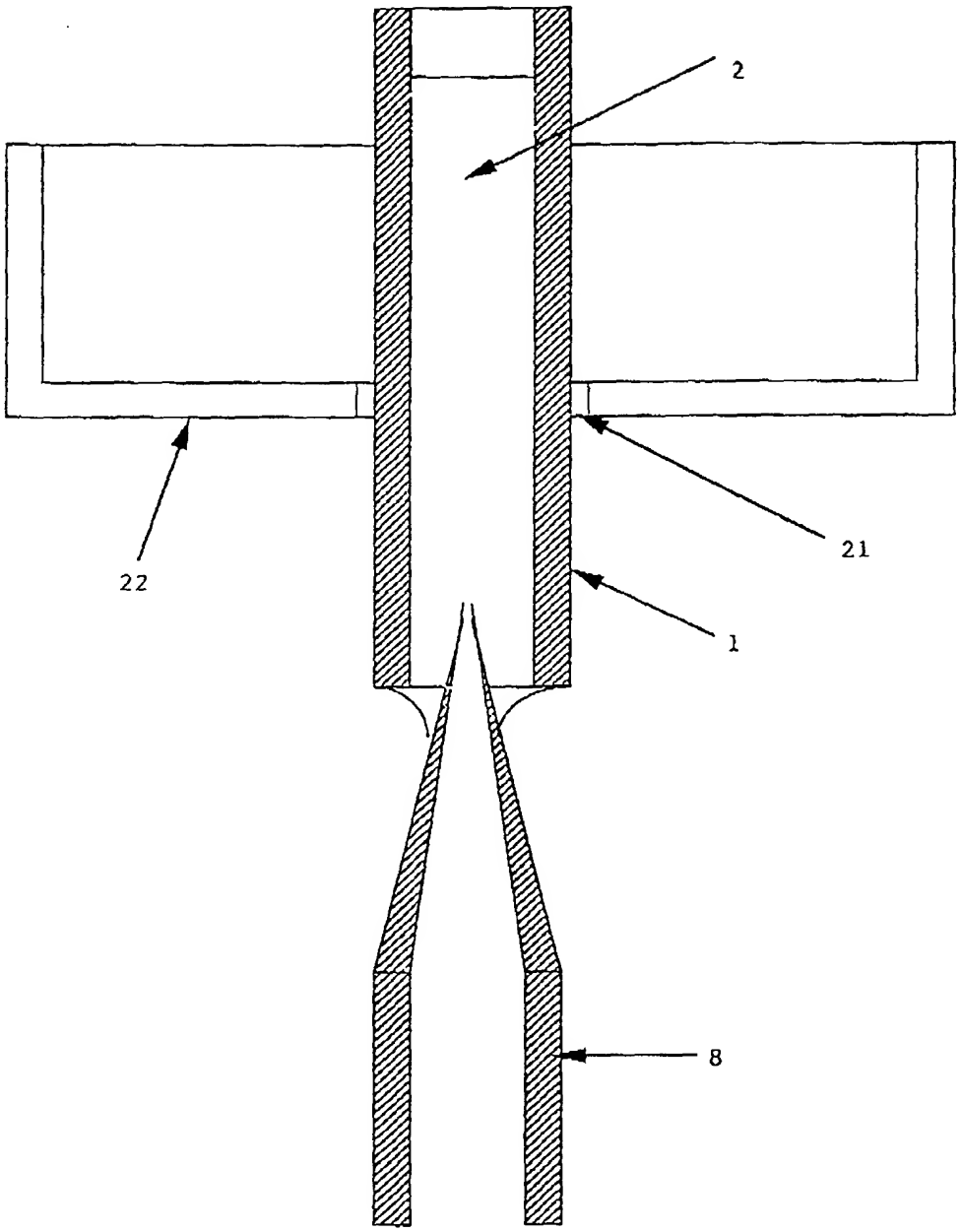


FIGURE 4

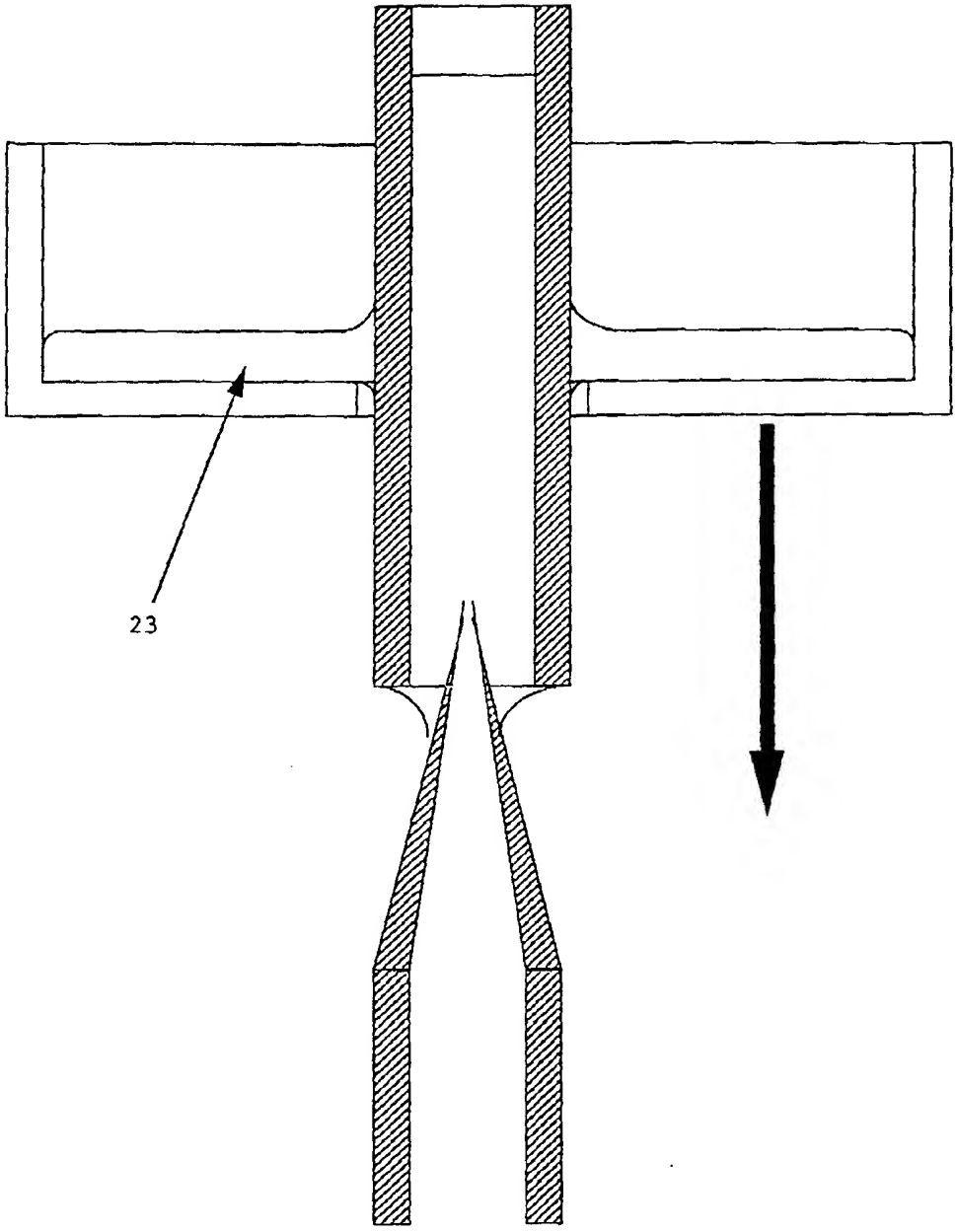


FIGURE 5

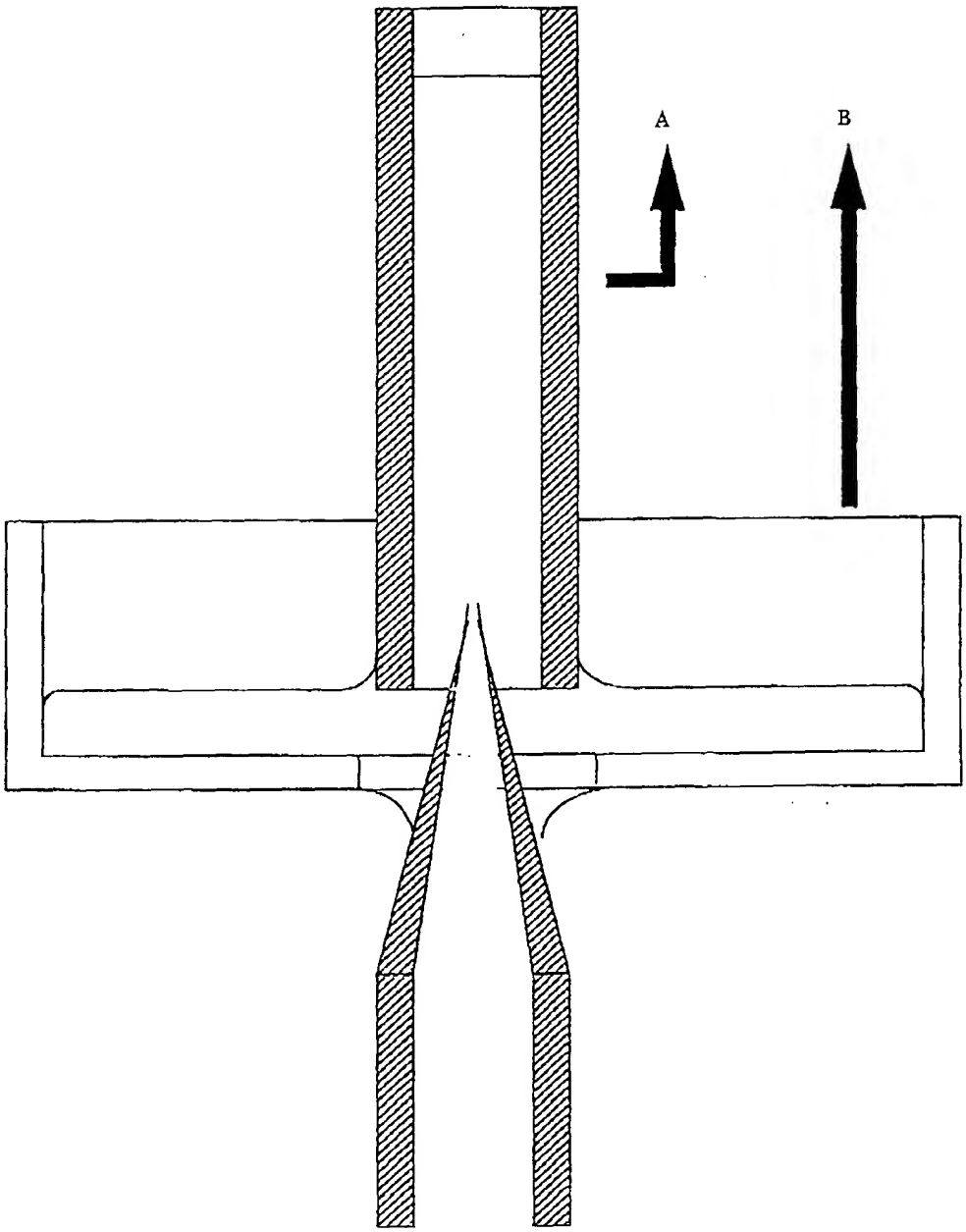


FIGURE 6

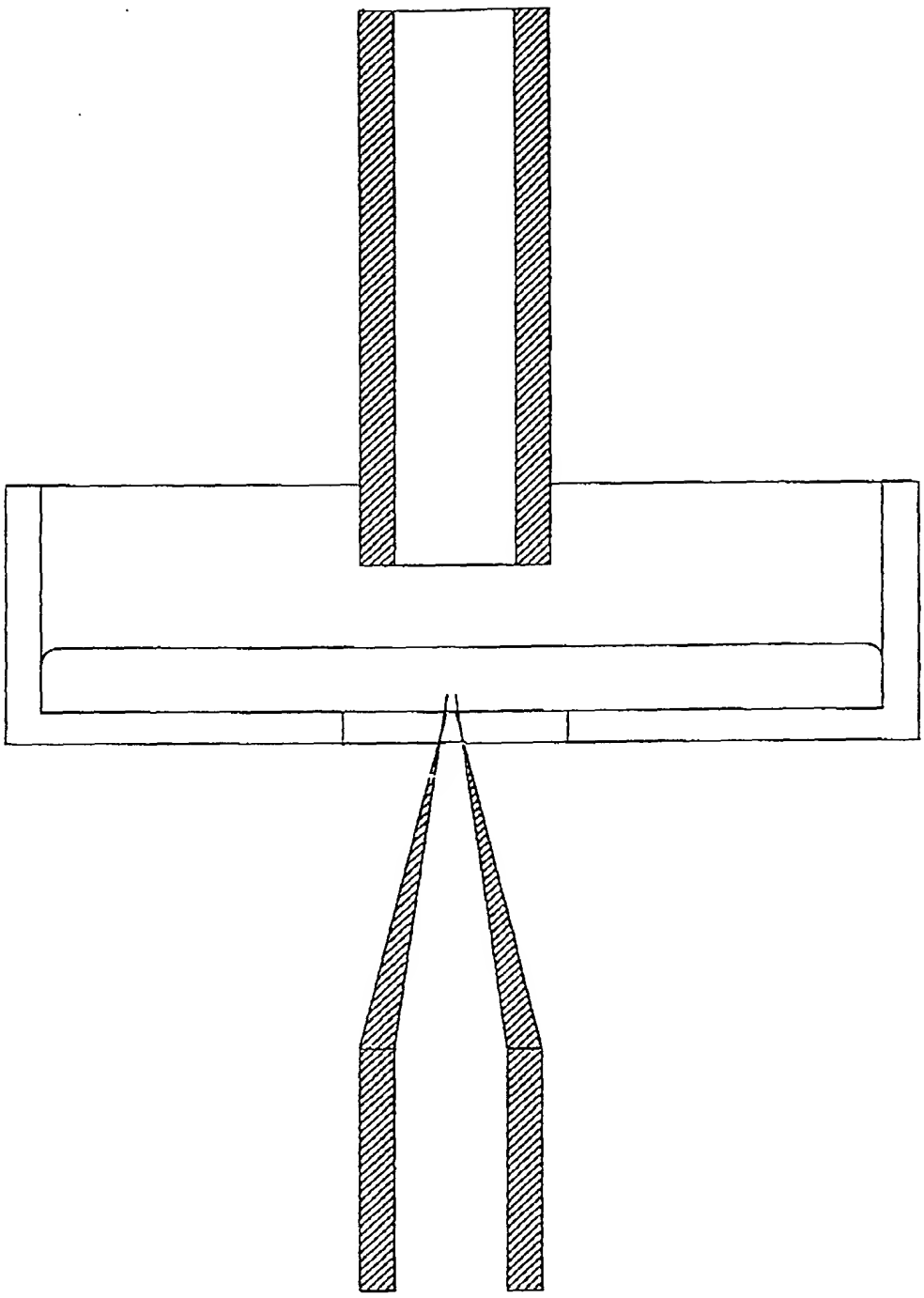


FIGURE 7

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